PROTEIN PURIFICATION USING ZEOLITE ADSORBENT

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I declare that this thesis entitled "*Protein Purification Using Zeolite Adsorbent"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :

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Date : 30 APRIL 2009

To my beloved father and mother, Mr Faisal and Madam Nooreini

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IN THE NAME OF ALLAH THE MOST GRACIOUS AND THE MOST MERCIFUL

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ABSTRACT

There has been much interest generated in the development of protein purification since many of modern biotechnology products can be produce from purified protein such as insulin, vaccines and food additives. The aim of this research is to study the most optimum condition to purify protein in order to produce a high purity protein with low cost by applying the principle adsorption process using zeolite adsorbent. Adsorption of Bovine Serum Albumin used as protein sample on Y, Beta and ZSM-5 zeolites at various pH values was carried out in sequence to investigate the effect of different pH value and different types of adsorbent on protein purification. Batch adsorption experiment was carried out by contacting the same amount of different zeolites synthesized with the Bovine Serum Albumin solution. Outcome of the experiment shows that maximum adsorption capacity occurred when Y zeolite was used as the adsorbent at pH 5 that is close to isoelectric point (pI) of Bovine Serum Albumin which is 4.7. This proves that adsorption capacity depends on the physicochemical properties of the zeolite as well as the pH of protein solution. The maximum adsorption on the zeolites tended to occur when the pH was at or just below the pI of the protein. At this pH, the protein sample is positively charged. Adsorption isotherms obtained on effect of different type of adsorbent at pH5 and effect of different pH on Y zeolite were found to be confirmed well to the ideal Langmuir model equation. In order to improve in the next study, other parameters such as protein size, temperature of the protein solution and protein concentration need to be considered as well.

ABSTRAK

Kini telah ramai yang menunjukkan minat di dalam penulenan protin apabila banyak produk bioteknologi yang moden dapat dihasilkan dari protin yang telah menjalani proses pemisahan seperti insulin, vaksin dan makanan tambahan. Tujuan kajian ini dijalankan adalah untuk mengenal pasti keadaan terbaik bagi penulenan protin dengan tujuan untuk menghasilkan protein yang mempunyai tahap ketulenan yang tinggi dengan kos pembiayaan yang rendah dengan cara mengaplikasikan prinsip proses penjerapan menggunakan zeolite. Penjerapan Bovine Serum Albumin yang digunakan sebagai sampel protin dijalankan ke atas zeolite Y, Beta dan ZSM-5 pada pelbagai nilai pH bertujuan untuk mengkaji kesan kepelbagaian nilai pH dan kesan penggunaan jenis zeolite yang berbeza ke atas proses penulenan protin. Kajian penjerapan secara 'batch' dijalankan dengan memasukkan jumlah zeolite yang seragam bersama-sama dengan larutan Bovine Serum Albumin. Keputusan daripada hasil kajian menunjukkan bahawa nilai maksimum penjerapan terjadi apabila zeolite Y digunakan pada pH 5, di mana pH tersebut merupakan pH yang paling hampir dengan nilai titik isoelektrik bagi Bovine Serum Albumin iaitu pada 4.7. Ini membuktikan bahawa kapasita penjerapan bergantung kepada sifat kimia-fizikal zeolite serta pH bagi larutan protin. Penjerapan maksima pada zeolite cenderung terjadi apabila nilai pH berada pada nilai titik isoelektrik atau nilai yang berhampiran dengan nilai titik isoelektrik bagi protin tersebut. Data keputusan yang diperoleh bagi penjerapan protin oleh zeolite didapati mematuhi konsep ideal Langmuir. Dalam rangka untuk mempertingkatkan data keputusan yang diperoleh untuk kajian selanjutnya, skop kajian lain seperti saiz protin, suhu bagi larutan protin dan kepekatan larutan protin perlu dipertimbangkan juga.

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LIST OF ABBREVIATIONS

BSA	-	Bovine Serum Albumin
DNA	-	Deoxyribonucleic acid
RNA	-	Ribonycleic acid
Da	-	Dalton
kDa	-	kiloDalton
SDS	-	Sodium Dodecyl Sulfate-polyacrylamide
NLLS	-	Non Linear least Squares
EF-AL	-	Extra Framework Aluminium
FAU	-	Faujasite
ELISA	-	Enzyme-Linked Immunosorbent Assay
pI	-	isoelectric point
RPM	-	revolutions per minute
Nm	-	nanometer
BEA	-	Beta
MFI	-	Pentasil

LIST OF SYMBOLS

G	-	Energy
H	-	Enthalpy
T	-	Temperature
x	-	Quantity adsorbed
m	-	Mass
P, P_0	-	Pressure
k,n	-	Empirical constant
θ	-	Theta
α	-	Alpha constant
q	-	Solute concentration
q_m	-	Langmuir isotherm parameter
C	-	Equilibrium concentration
K_d	-	Langmuir adsorption parameter
R^2	-	Regression value
V, V_m	-	Adsorbed gas quantity

CHAPTER 1

INTRODUCTION

1.1 Introduction

Proteins are large organic compounds made of amino acids formed in a linear chain. They are joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. The study of proteins and their function is central to understanding both cells and organisms. There are a few reasons why proteins are important in biology which are; protein serve as a catalyst that maintain metabolic processes in the cell and also they serve as structural elements both within and outside the cell.

Various chromatographic methods such as ion exchange, affinity and hydrophobic interaction have been used for the separation of various kinds of proteins, but these resins have many difficulties. Some of them are very weak chemically and physically, for example, strong acid and alkali or high and low temperature will affect the process. Many proteins that are released and solubilized from biological structural matrices become very unstable and consequently, are essentially irrelevant from a biochemical perspective. Thus, a study had been conducted to prove those zeolites are able to adsorb protein on their surface with the intention to purify the protein (C.Hiroyuki, 2002).

Protein purification is the separation of a specific protein from contaminants in a manner that produces a useful end product. Effective separation and purification of proteins have been an important issue in the biomedical and pharmaceutical industries. Protein adsorption has been developed in biotechnology to achieve highly efficient with an economical separation processes. In many cases, proteins which have similar physical and chemical properties need to be separated, and thus highly selective adsorbents are desired. Micro porous molecular sieves, such as zeolite Y, ZSM-5 and Beta zeolite, have played important roles in acid catalysis because of their peculiar pore structures and strong intrinsic acidities.

It is challenging to develop simple, low cost, and scalable methods for large scale recombinant protein purification with a reasonable separation solution. Low cost protein purification methods are in high demand for mass production of low selling price enzymes that play an important role in the upcoming bio-economy (Jiong Hong, 2008). The surface adsorption of proteins on heterogeneous supports is important in a wide variety of medical and biochemical implant in a living body, the cell growth in a culture, or the functionality of a biosensor.

Zeolites are inorganic materials which have a highly ordered structure and can be synthesized with a nanocrystalline size. Zeolites also offer interesting characteristics, such as mechanical and chemical resistance as well as high surface area. On the other hand, zeolites are also known to be stale both in wet and dry conditions and well tolerated by microorganism, and therefore normally compatible with biochemical analyses.

Zeolites due to their low toxicity and high compatibility are considered new biomaterials for medical applications (Adalgisa Tavolaro, 2006). In this work, the protein adsorption on several type of zeolite at various pH were carried out using Bovine Serum Albumin (BSA) as model proteins. The interaction between protein and zeolite adsorbent will be studied using UV-VIS Spectrophotometer to monitor the purified protein solution. It is believes that this protein separation method can be scaled up easily because it is based on simple solid-liquid unit operations.

1.2 Problem Statement

A pure sample of protein is required to generate antibodies, conduct binding assays and study structure. Yet, the target protein must first be isolated; the debris, salts, and reagents washed away, the amount of protein quantified and also the sample concentrated.

At present, protein purification is a challenge because, in addition to the particular protein that is meant to be purified, the protein's cell contains several thousand other proteins along with nucleic acids (DNA and RNA), polysaccharides, lipids as well as small molecules. By purifying a protein, a specific protein will be separated from contaminants in a manner that it will produce a useful end product. However, a highly efficient method with a cost-effective way has yet to be developed. This study focuses particularly on the principle of protein adsorption using pure adsorbent particle in order to purify the protein sample.

1.3 Objective of Study

The objective of this research is to study the most optimum condition to purify protein in order to produce a high yield and high purity protein with low cost by applying the principle of adsorption process using zeolite adsorbent.

1.4 Scope of Study

In sequence to accomplish the objective, the following scopes have been identified:

1. Effect of different pH value on protein purification
2. Effect of different type of adsorbent on protein purification

For the first parameter, the effect of pH on the process of protein purification applying adsorption principle using zeolite adsorbent is being considered at four different pH's namely 3, 4, 5, and 7. The samples at various pH will be prepared and were continuously shaken at room temperature until it is in equilibrium condition. For the next parameter, the same experiment will be carried out by using different types of adsorbent which are zeolite Y, Beta and ZSM-5 in order to monitor the effect of different type of adsorbent used.

CHAPTER 2

LITERATURE REVIEW

2.1 Protein

The word protein was first mentioned in a letter sent by the Swedish chemist Jöns Jakob Berzelius to Gerhardus Johannes Mulder on July 10, 1838. Proteins were recognized as a distinct class of biological molecules in the eighteenth century by Antoine Fourcroy and others, distinguished by the molecules' ability to coagulate or flocculate under treatments with heat or acid. During that time, examples of protein included albumin from egg whites, blood, serum albumin, fibrin, and wheat gluten. Dutch chemist Gerhardus Johannes Mulder carried out elemental analysis of common proteins and found that nearly all proteins had the same empirical formula. The term "protein" to describe these molecules was proposed in 1838 by Mulder's associate Jöns Jakob Berzelius. Mulder went on to identify the products of protein degradation such as the amino acid leucine for which he found a molecular weight of 131 Da.

Proteins are large organic compounds made of amino acids formed in a linear chain. They are joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. Proteins are large biological molecules with molecular weight up to few million Daltons. For convenience, the protein weight is measured in thousands Daltons or kilo Daltons (kDa).

Proteins are made of amino acids linked into linear chains, called polypeptide chains. Amino acids links between each other by peptide bonds - this peptide bond is formed between the carboxyl and amino groups of neighbouring amino acids.

Proteins are formed by one or several polypeptide chains. The sequence of the polypeptide chain is defined by a gene with genetic code. There are only 20 standard amino acids are exists in living organism. Sometimes these amino acids are chemically modified in the protein after protein synthesis. In total the number of different proteins, which it is possible to produce from 20 amino acids is enormous. For example for 10 amino acid sequence it is possible to have 20^{10} different sequences, which is approximately equal to 10^{13} or 10 trillions of different structures. The study of proteins and their function is central to understanding both cells and organisms. There are a few reasons why proteins are important in biology which are; protein serve as a catalyst that maintain metabolic processes in the cell and also they serve as structural elements both within and outside the cell.

Protein structure is essential for correct function because it allows molecular recognition. For example, enzymes are proteins that catalyze biochemical reactions. The function of an enzyme relies on the structure of its active site, a cavity in the protein with a shape and size that enable it to fit the intended substrate very snugly. It also has the correct chemical properties to bind the substrate efficiently. The active site also contains certain amino acids that are involved in the chemical reaction catalyzed by the enzyme.

Although not all proteins are enzymes, but still, all in some way rely on molecular recognition in order to perform their functions. Transport proteins such as hemoglobin must recognize the molecules they carry, receptors on the cell surface must recognize particular signaling molecules, and transcription factors must recognize particular DNA sequences and antibodies must recognize specific antigens. The functional integrity of the cell depends critically on protein-protein interactions, particularly on the formation of multi-protein complexes.

Protein is the major component of living organisms thus it formed a wide variety of essential functions in cells. A number of products that are being produced by modern companies specified in biotechnologies use protein in their process. These proteins that are being used may be drugs such as insulin or they might be in the formed of molecular tools that allows researchers to use it as an enzymes.

Serum albumin is one of the most widely studied proteins and is the most abundant protein in plasma. Various researchers have studied the structure and properties of serum albumin and its interaction with other proteins in order to understand how serum albumin affects the functionality of foods in which they have been included as well as novel applications. The latter reason led to the study of the interaction between soluble wheat protein and bovine serum albumin.

2.1.1 Structure of Protein

Generally, proteins contain from a range of 50 to 1000 amino acid residues per polypeptide chain. A peptide bond is an amide bond formed by the reaction of an α-amino group (NH_2) of one amino acid with the carboxyl group (COOH) of another, as shown below in Figure 2.1

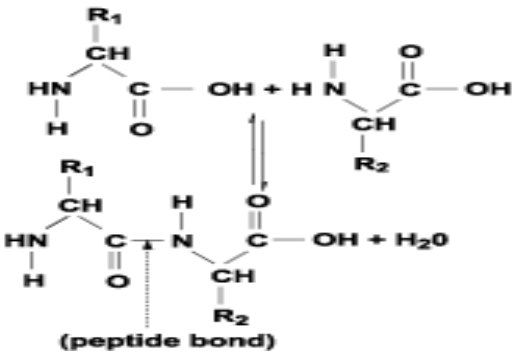

Figure 2.1: Peptide bond

Proteins are not entirely rigid molecules. In addition to these levels of structure, proteins may shift between several related structures while they perform their biological function. In the context of these functional rearrangements, these tertiary or quaternary structures are usually referred to as "conformations", and transitions between them are called conformational changes. Proteins can be informally divided into three main classes, which correlate with typical tertiary structures: globular proteins, fibrous proteins, and membrane proteins.

The structures build from 2 to 100 amino acids with molecular weight up to 10 kDa are usually called peptides. Longer polypeptide structures are classified as proteins. Some other classifications are appeal to the conformations stability of the amino acid chain. In this classification, peptides have many different conformations and can randomly change them, whereas proteins are structurally rigid with only one preferable conformation.

Based upon chemical composition, proteins are divided into two major classes: simple proteins, which are composed of only amino acids, and conjugated proteins, which are composed of amino acids and additional organic and inorganic groupings, certain of which are called prosthetic groups. Conjugated proteins include glycoproteins, which contain carbohydrates; lipoproteins, which contain lipids; and nucleoproteins, which contain nucleic acids.

The structure of proteins is a complex one which is divided into 4 parts. They are primary, secondary, tertiary and quaternary structures. For primary structure, this structure is responsible for the function of a protein. This structure is composed of various amino acids held together by peptide bonds. Proteins may have 1 or more polypeptide chains. Each polypeptide in a protein has amino acids linked with each other in a specific sequence and it is this sequence of amino acids that is said to be the primary structure of that protein. A protein containing a total of 100 amino acids residues is a very small protein, yet 20 different amino acids can be combined at one time in $(20)^{100}$ different ways.

The physical interaction of sequential amino-acid sub-units results in a so-called secondary structure, which often can either be a twisting of the polypeptide chain approximating a linear helix (α-configuration), or a zigzag pattern (β-configuration). Most globular proteins also undergo extensive folding of the chain into a complex three-dimensional geometry designated as tertiary structure. Many globular protein molecules are easily crystallized and have been examined by X-ray diffraction, a technique that allows the visualization of the precise three-dimensional positioning of atoms in relation to each other in a crystal.

The three dimensional structure of a protein is known as tertiary structure of a protein. This is a compact structure which means further folding of the secondary structure. It coils and folds in such a way that the hydrophobic side chains are held interior and the hydrophilic groups are held outside. This arrangement gives stability to the molecule. The tertiary structure is maintained by hydrogen bonds, disulfide bonds, ionic bonds and hydrophobic interactions. This structure brings distant amino acid side chains nearer. Two major molecular shapes are found which are fibrous and globular. The fibrous proteins such as silk collagen and a-keratins have large helical content and have rod like rigid shape and are insoluble in water.

In globular proteins such as hemoglobin the polypeptide chains consist partly of helical sections which are folded about the random cuts to give it a spherical shape. The primary, secondary and tertiary and quaternary levels of hemoglobin structure are given in figure at the next page.

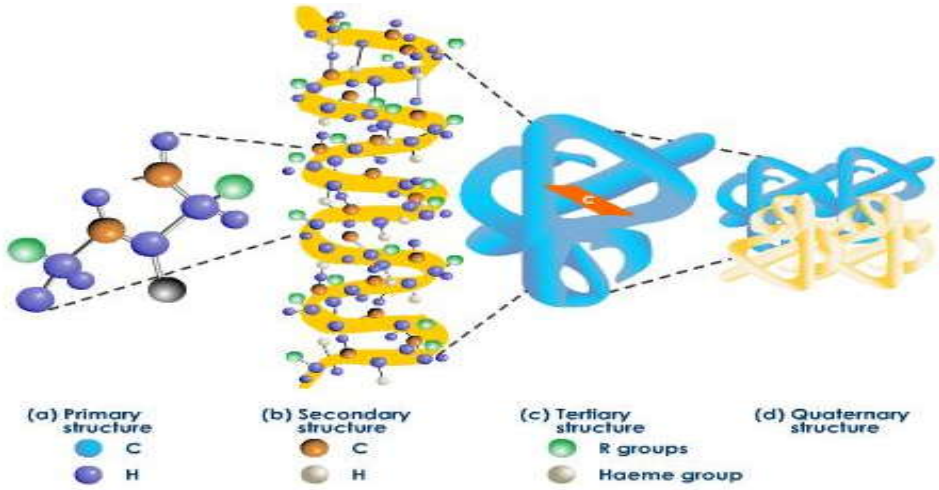

Figure 2.2: Primary, secondary, tertiary and quaternary structures of hemoglobin

2.1.2 Protein Biosynthesis

Protein biosynthesis is a process in which cells build proteins. The term is sometimes used to refer only to protein translation but more often it refers to a multi-step process, beginning with amino acid synthesis and transcription which are then used for translation. Protein biosynthesis, although very similar, differs between prokaryotes and eukaryotes. The processes by which proteins are synthesized biologically have become one of the central themes of molecular biology. The sequence of amino acid residues in a protein is controlled by the sequence of the DNA as expressed in messenger RNA at ribosomes.

Protein synthesis is the creation of proteins using DNA and RNA. Proteins can often be synthesized directly from genes by translating mRNA. When a protein is harmful and needs to be available on short notice or in large quantities, a protein precursor is produced. A pro-protein is an inactive protein containing one or more inhibitory peptides that can be activated when the inhibitory sequence is removed by proteolysis during posttranslational modification.